

What is Claimed is:

1. A plant comprising transformed plant cells, said transformed plant cells containing a heterologous nucleic acid construct comprising, in the 5' to 3' direction:

- 5 (a) a promoter operable in said plant cells,
- (b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the Rb binding region, whereby binding of said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein; and
- 10 (c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith,

wherein expression of said mutant AL1 protein increases resistance of said plant to infection by at least one geminivirus, compared to a non-transformed control.

2. A plant according to claim 1, where said nucleic acid sequence encodes a trans-dominant negative mutant AL1 protein.

3. A plant according to claim 2, wherein said trans-dominant negative mutant AL1 protein has a mutation in the oligomerization domain domain.

4. A plant according to claim 2, wherein said trans-dominant negative mutant AL1 protein has a mutation in the DNA cleavage domain.

5. A plant according to claim 2, wherein said trans-dominant negative mutant AL1 protein has a mutation in the ATPase domain.

6. A plant according to claim 1, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

5 7. A plant according to claim 1, wherein said plant has increased resistance to a geminivirus selected from the group consisting of tomato golden mosaic virus, tomato mottle virus, tomato yellow leaf curl virus, tomato leaf curl virus, African cassava mosaic virus, Indian cassava mosaic virus, potato yellow mosaic virus, bean golden mosaic virus, bean dwarf mosaic virus, squash leaf curl virus, Texas pepper virus, cotton leaf curl virus and beet curly top virus.

8. A plant according to claim 1, wherein said promoter is constitutively active in said plant.

9. A plant according to claim 1, which plant is selected from the group consisting of tomato, cassava, potato, bean, squash and beet.

10. A plant according to claim 1, wherein said plant is of the family Solanaceae.

11. A plant according to claim 1, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:8.

12. A plant according to claim 2, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

13. A plant according to claim 6, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

14. A plant according to claim 11, wherein said plant is a tomato plant and has increased resistance to tomato golden mosaic virus (TGMV).

15. A plant comprising transformed plant cells, said transformed plant cells containing a heterologous nucleic acid construct comprising, in the 5' to 3' direction:

- 5 (a) a promoter operable in said plant cells,  
(b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the oligomerization domain to produce a trans-dominant negative mutant AL1 protein; and  
(c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith,

10 wherein expression of said mutant AL1 protein increases resistance of said plant to infection by at least one geminivirus, compared to a non-transformed control.

16. A plant according to claim 15, wherein said nucleic acid sequence further comprises a mutation in the Rb binding region, whereby binding of said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein.

17. A plant according to claim 15, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

18. A plant according to claim 15, wherein said plant has increased resistance to a geminivirus selected from the group consisting of tomato golden mosaic virus, tomato mottle virus, tomato yellow leaf curl virus, tomato leaf curl virus, African cassava mosaic virus, Indian cassava mosaic virus, potato yellow mosaic virus, bean  
5 golden mosaic virus, bean dwarf mosaic virus, squash leaf curl virus, Texas pepper virus, cotton leaf curl virus and beet curly top virus.

19. A plant according to claim 15, wherein said promoter is constitutively active in said plant.

20. A plant according to claim 15, which plant is selected from the group consisting of tomato, cassava, potato, bean, squash and beet.

21. A plant according to claim 15, wherein said plant is of the family Solanaceae

22. A plant according to claim 15, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

23. A plant according to claim 16, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:8.

24. A plant according to claim 15, wherein said plant is a tomato plant and has increased resistance to tomato golden mosaic virus (TGMV).

25. A method of combating geminivirus infection in an agricultural field, comprising planting the field with a crop of plants according to claim 1.

26. A method of combating geminivirus infection in an agricultural field, comprising planting the field with a crop of plants according to claim 15.

27. A method of making a transgenic plant having increased resistance to geminivirus infection, said method comprising:

providing a plant cell capable of regeneration;

transforming said plant cell with a DNA construct comprising, in the 5' to 3' direction, (a) a promoter operable in said plant cells, (b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the

Rb binding region, whereby binding of said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein; and (c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith; and then

- 5       regenerating a transgenic geminivirus-resistant plant from said transformed plant cell, wherein expression of said mutant AL1 protein increases resistance of said plant to infection by at least one geminivirus, compared to a non-transformed control.

28. A method according to claim 27, where said nucleic acid sequence encodes a trans-dominant negative mutant AL1 protein.

29. A method according to claim 28, wherein said trans-dominant negative mutant AL1 protein has a mutation in a domain selected from the group consisting of the oligomerization domain, the DNA cleavage domain and the ATPase domain.

30. A method according to claim 28, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

31. A method according to claim 27, wherein said promoter is constitutively active in said plant.

32. A method according to claim 27, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:8.

33. A method according to claim 28, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

34. A method according to claim 27, wherein said plant cell resides in a plant tissue capable of regeneration.

35. A method according to claim 27, wherein said transforming step is carried out by bombarding said plant cell with microparticles carrying said expression cassette.

36. A method according to claim 27, wherein said transforming step is carried out by infecting said cells with an *Agrobacterium tumefaciens* containing a Ti plasmid carrying said expression cassette.

37. A method of making a transgenic plant having increased resistance to geminivirus infection, said method comprising:

providing a plant cell capable of regeneration;

transforming said plant cell with a DNA construct comprising, in the 5' to 3' direction, (a) a promoter operable in said plant cells, (b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the oligomerization domain to produce a trans-dominant negative mutant AL1 protein; and (c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith; and then

regenerating a transgenic geminivirus-resistant plant from said transformed plant cell, wherein expression of said mutant AL1 protein increases resistance of said plant to infection by at least one geminivirus, compared to a non-transformed control.

38. A method according to claim 37, wherein said nucleic acid sequence further comprises a mutation in the Rb binding region, whereby binding of said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein.

39. A method according to claim 37, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

40. A method according to claim 37, wherein said promoter is constitutively active in said plant.

41. A method according to claim 37, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

42. A nucleic acid construct comprising an expression cassette, which construct comprises, in the 5' to 3' direction:

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- (a) a promoter operable in a plant cell,
  - (b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the Rb binding region, whereby binding of said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein; and
  - (c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith.

43. A nucleic acid construct according to claim 42 carried by a plant transformation vector.

44. A nucleic acid construct according to claim 42, where said nucleic acid sequence encodes a trans-dominant negative mutant AL1 protein.

45. A nucleic acid construct according to claim 42, wherein said trans-dominant negative mutant AL1 protein has a mutation in a domain selected from the group consisting of the oligomerization domain, the DNA cleavage domain, and the ATPase domain.

46. A nucleic acid construct according to claim 42, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

47. A nucleic acid construct according to claim 42, wherein said promoter is constitutively active in said plant.

48. A nucleic acid construct according to claim 42, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:8.

49. A method according to claim 44, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

50. A nucleic acid construct comprising an expression cassette, which construct comprises, in the 5' to 3' direction:

- (a) a promoter operable in said plant cells,
- (b) a nucleic acid sequence encoding a mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and comprising a mutation in the oligomerization domain to produce a trans-dominant negative mutant AL1 protein; and
- (c) a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith.

51. A DNA construct according to claim 50 carried by a plant transformation vector.

52. A DNA construct according to claim 50, wherein said nucleic acid sequence further comprises a mutation in the Rb binding region, whereby binding of



said mutant AL1 protein to a plant Rb protein is reduced compared to binding which would occur in the presence of a wild-type AL1 protein.

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53. A DNA construct according to claim 50, wherein said nucleic acid sequence encodes an AL1 protein with increased repression of transcription from the AL1 promoter, compared to a wild-type AL1 protein.

54. A DNA construct according to claim 50, wherein said promoter is constitutively active in said plant.

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55. A DNA construct according to claim 50, wherein said nucleic acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

56. A method of producing nucleic acid constructs useful in conferring increased geminivirus-resistance to plants, comprising:

- 5 a) screening mutants of a geminivirus AL1 protein, where the mutation is located in the Rb binding domain, to identify mutations that suppress the ability of the AL1 protein to bind to a plant Rb protein;
- 10 b) preparing a nucleic acid construct comprising, in the 5' to 3' direction, a promoter operable in a plant cell, a nucleic acid sequence encoding said mutant AL1 protein, said nucleic acid sequence further having a mutation that suppresses geminivirus replication compared to that which would occur in the presence of a wild-type AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith.

57. A method of producing nucleic acid constructs useful in conferring increased geminivirus-resistance to plants, comprising:

a) screening mutants of a geminivirus AL1 protein, where the mutation is located in the oligomerization binding domain, to identify trans-dominant AL1 mutants;

5 b) preparing a nucleic acid construct comprising, in the 5' to 3' direction, a promoter operable in a plant cell, a nucleic acid sequence encoding said mutant AL1 protein, said nucleic acid sequence located downstream from said promoter and operatively associated therewith, and a termination sequence positioned downstream from said nucleic acid sequence and operatively associated therewith.

58. Seed or progeny of a plant according to claim 1, which seed or progeny has inherited said nucleic acid sequence encoding a mutant AL1 protein.

59. Seed or progeny of a plant according to claim 15, which seed or progeny has inherited said nucleic acid sequence encoding a mutant AL1 protein.

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